Kidney International, Vol. 10 (1976), p. 364–372

Enhanced end-organ responsiveness of the uremic kidney to the natriuretic factor

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Enhanced end-organ responsiveness of the uremic kidney to the natriuretic factor. In chronic renal disease, the addition of a fixed quantity of Na to the extracellular fluid (ECF) will evoke a natriuretic response per nephron which is inversely proportional to the glomerular filtration rate (GFR). One factor that could contribute to this "magnification" phenomenon is an increased sensitivity of residual nephrons to physiologic natriuretic forces. The present studies were designed to examine this possibility. Natriuretic urine fractions from uremic patients, infused into one renal artery of normal rats, produced a small but significant unilateral natriuresis. Infusion of the same fractions in identical amount into remnant kidneys of stage II nonuremic rats (i.e., rats with a contralateral normal kidney *in situ*) produced a natriuresis in the remnant kidney only which was equivalent to that observed in the normal kidneys. The i.v. infusion of natriuretic fractions into stage II rats produced comparable increments in the fractional excretion of sodium (FE_{Na}) bilaterally. However, when the natriuretic fractions were infused into remnant kidneys of stage III rats (no contralateral kidney), ΔFE_{Na} was significantly greater than in the foregoing groups. Because stage III rats have increased control values for FE_{Na}, baseline FE_{Na} was increased to an equivalent level in normal rats by unilateral renal denervation. Natriuretic factor was administered into the ipsilateral renal artery. Although the natriuretic response was increased, it was significantly less than in the stage III remnant kidneys. The data support the view that the uremic state per se is associated with an enhanced responsiveness of the residual nephrons to the natriuretic factor found in the urine (and blood) of uremic patients.

Réponse accrue du rein urémique au facteur natriurétique. Au cours de l'insuffisance rénale chronique, l'addition d'une quantité déterminée de sodium (Na) au liquide extracellulaire (ECF) détermine une réponse natriurétique par néphron qui est inversement proportionnelle au débit de filtration glomérulaire (GFR). Un facteur qui pourrait contribuer à ce phénomène de "magnification" est l'augmentation de la sensibilité des néphrons résiduels aux forces natriurétiques. Ce travail a pour but d'étudier cette possibilité. Les fractions natriurétiques de l'urine de sujets urémiques, injectées dans une artère rénale de rat normal, déterminent une natriurèse unilatérale minime mais significative. L'injection de la même quantité des mêmes fractions dans le rein lésé de rats du stade II non urémiques (rats avec un rein controlatéral en place) produit une natriurèse dans le rein lésé seulement, natriurèse équivalente à celle observée dans les reins normaux. L'administration intra-veineuse de fractions natriurétiques à des rats du stade II produit une augmentation bilatérale comparable de FE_{Na}. Cependant, quand les fractions natriurétiques sont injectées dans les reins lésés des animaux du stade III (pas de rein controlatéral), ΔFE_{Na} est significativement plus grand que dans le groupe précédent. Puisque les rats du stade III ont des valeurs contrôles de FE_{Na} augmentées, FE_{Na} basal a été amené à un niveau comparable chez des rats normaux par la dénervation rénale unilatérale. Le facteur natriurétique a été administré dans l'artère rénale ipsilatérale. Bien que la réponse natriurétique ait été plus grande elle est restée significativement inférieure à celle obtenue dans les reins lésés des animaux du stade III. Ces résultats sont en faveur de l'idée que l'état urémique par lui même est associé à une augmentation de la capacité de réponse des néphrons résiduels au facteur natriurétique qui existe dans l'urine (et le sang) des malades urémiques.

Received for publication February 24, 1976;
and in revised form May 18, 1976.

The biologic control system regulating sodium excretion by the kidney undergoes a progressive "magnification" response throughout the course of chronic renal disease [1–4]. This adaptation is characterized by an increase in fractional sodium excretion (FE_{Na}) which is inversely proportional to the glomerlular filtration rate (GFR) and which occurs without a concomitant increase in sodium intake. It is by virtue of this transformation in nephron function that the uremic patient continues to maintain external sodium balance as GFR falls to very low concentrations without the necessity for progressive salt restriction.

How the magnification response is mediated remains a matter of speculation. One possibility is that, as GFR falls, the same perturbation of extracellular fluid (ECF) volume (such as the minimal expansion produced by the amount of NaCl in a normal diet) evokes an ever-increasing degree of activation of the sodium control system, and in some manner affects the release of an increasing quantity of "natriuretic forces." The latter would act on the residual nephrons inhibiting net sodium reabsorption. A second possibility is that the sensitivity of the residual nephrons to one or more of the "natriuretic forces" increases adaptively as GFR falls. The two mechanisms are in no way mutually exclusive.

Among the presumed natriuretic forces in chronic uremia is a circulating inhibitor of sodium transport

which is found in both the serum and urine of chronically uremic patients and dogs [5–7]. The same factor exists in the urine of normal dogs with chronic ECF volume expansion undergoing mineralocorticoid hormone "escape" [8], and in normal young men undergoing "water immersion" natriuresis [9]. There appears to be a correlation between the activity of a natriuretic factor and the concurrent requirements for sodium excretion per nephron in the subjects or animals from which the test materials have been obtained [6–8], and we believe, on the basis of cumulative evidence, that this natriuretic factor could be among the physiologic mediators of sodium excretion. The rate of production of this natriuretic factor appears to be increased in uremia in the absence of an increased intake of NaCl as evidenced by increased plasma concentrations at the same time that urinary excretion rates are increased [5, 6]. Previous studies, however, have not examined whether an altered responsiveness of the kidney plays an additional role in the magnification response of uremia. The present study represents an effort to evaluate the possibility that the sensitivity of the kidney to the natriuretic factor increases in uremia.

Methods

Preparation of urine fractions. Timed urine collections, either 12 or 24 hr in duration, were obtained from patients with advanced chronic renal failure of various etiologies and from normal subjects. Endogenous creatinine clearance was less than 15 ml/min in all of the patients. All subjects, whether uremic or normal, were on an average (i.e., unrestricted) salt intake, and all were judged on clinical grounds to be in external sodium balance. None of the uremic patients were being maintained with intermittent hemodialysis. The technique of processing the urine samples has been described previously [6]. In brief, the original 12- or 24-hr urine collection was concentrated by lyophilization and 25 ml of the concentrate, representing the equivalent of a 5-hr urine sample, was filtered through Sephadex G25. Elution was performed with a solution of 10 mM ammonium acetate at a pH of 6.8. The partition of the effluent solution and the fraction used for study have been described in detail previously [6]. Each fraction employed in the present study was pretested using a standard bioassay system [5, 6]. According to the criteria previously established [5, 6], only uremic fractions which were "natriuretic" and normal fractions which were "nonnatriuretic" were employed in the present studies.

Natriuretic fractions. Twenty-five experiments were performed using the natriuretic fractions obtained from nine uremic patients. Each of the nine fractions was infused into the left renal artery of nine uremic (i.e., stage III) hydropenic rats with a solitary "remnant" kidney. An identical volume of each of six of these fractions was infused into the renal artery of six normal hydropenic (i.e., stage I) rats. Three of these fractions were also infused into the renal artery of the remnant kidney of hydropenic nonuremic (i.e. stage II) rats in which the contralateral kidney had not been removed. Finally, seven of the nine fractions were infused into the left renal artery of normal rats subjected to left renal denervation at least three hours prior to the infusion. Two of the above fractions plus additional fractions from three other patients were infused intravenously into five stage II rats. The effects on the remnant kidney were compared with those simultaneously produced in the contralateral normal organ. These animals received 0.2% NaCl in lieu of drinking water for two days prior to the experiment as described previously [5, 6].

Nonnatriuretic fractions. Fourteen experiments were performed using nonnatriuretic fractions from ten normal subjects. Five of the fractions were infused into the left renal artery of stage I hydropenic rats. Four of these five fractions were also infused into the left renal artery of normal rats subjected to left renal denervation. The other five fractions were infused into the solitary renal artery of stage III rats.

Surgical procedures and method of bioassay. The "remnant kidneys" in the stage II and stage III animals were produced by infarcting approximately 75% of the left kidney. This was accomplished by ligating second and third order branches of the renal artery. In the stage III rats, the right kidney was removed in a separate surgical procedure. Unilateral renal denervation was performed as described below.

On the day of the experiment, each test animal was anesthetized lightly with ether and its left renal artery was exposed through a longitudinal flank incision. Retroperitoneal fat and connective tissue were carefully separated with moist cotton-tipped applicators and the renal artery was cannulated using a bent 33-gauge needle with a PE-10 polyethylene extension. The technique of unilateral renal arterial cannulation has been described previously [10]. The polyethylene catheter was sutured to the subcutaneous tissues of the back and brought to the exterior at the base of the tail. The test solutions were infused through the intraarterial catheter in the manner described below.

Unilateral renal denervation was performed by exposing the left renal artery through a flank incision, dissecting the surrounding tissue, gently scraping and severing all visible nerves and then applying lidocaine topically to the exposed renal pedicle.

In all rats with two kidneys (stage I, stage II, and

stage I with left renal denervation), urine from the left kidney was obtained through a PE-50 catheter with a tapered tip. The latter was introduced into the ureter via a midline abdominal incision. Urine from the right kidney was obtained through a bladder catheter. In the stage III animals, urine was collected via a bladder catheter. Complete emptying of the bladder was facilitated in the manner described previously [5]. The jugular vein and femoral artery were also cannulated routinely. The rats then were placed in a standard plastic (Lucite) restraining chamber and were allowed to recover from the anesthetic. All studies were carried out after a recovery period of approximately one hour with the animals fully awake. A priming dose of 3 μCi ^{14}C-inulin and 5 μCi of ^{3}H-para-aminohippurate (^{3}H-PAH) in a volume of 1 ml of 0.9% NaCl was administered intravenously over a five-min interval. A sustaining infusion containing 1.5 μCi/ml of ^{14}C-inulin and 2.5 μCi/ml of ^{3}H-PAH in a vehicle of 0.45% NaCl was infused at a rate of 20 μl/min throughout the experiment using a Harvard syringe pump. A one- to two-hour period of equilibration was allowed after the initiation of the sustaining infusion for stabilization of the rate of urine flow. Three 15-min control clearance periods were then obtained. During the equilibration period and the three control periods, 0.9% NaCl containing 4 mM KCl (pH 7.4) was infused into the renal arterial catheter at a rate of 20 μl/min. At the completion of the third control period, the intraarterial infusate was changed to one containing the experimental fraction. (The sodium and potassium concentrations of the latter were adjusted to 150 and 4 mEq/liter, respectively; the pH was titrated to 7.4.) The rate of infusion was maintained at 20μl/min. Three additional 15-min experimental clearance periods were then obtained. Finally, in a number of experiments, the test solution containing the unknown fraction was replaced by the original NaCl-KCl solution, and three follow-up 15-min clearance periods were obtained. The quantity of fraction administered during the three experimental periods was equivalent to the total amount harvested from a two-hr urine sample.

In five of the experiments on stage II rats, the same amount of fraction was administered intravenously over a five-min period via the jugular vein catheter. Urine collections were made from both kidneys as described above.

Blood was drawn at the midpoint of each urine collection period via the femoral arterial cannula. Blood pressure was measured using a mercury manometer just prior to obtaining the blood samples. If the arterial catheter did not function properly, venous blood was obtained from the tail.

Samples of plasma (25 μl) and urine (50 μl) were pipetted into plastic counting vials containing ten ml of Aquasol (New England Nuclear Co.), and ^{14}C and ^{3}H activity were counted using a liquid scintillation spectrometer (Packard Tricarb, Model 3214, Packard Instrument Co., Inc., Downes Grove, IL). At least 10,000 counts were obtained on each samples. Sodium and potassium concentrations were measured on both serum and urine samples using a flame photometer (Model 43, Instrumentation Laboratory, Inc., Lexington, MA).

Two prospective criteria were established for the acceptability of an experiment: *1*) In the rats in which one or both kidneys had a normal complement of nephrons, a GFR of greater than 0.7 ml/min per kidney was required. *2*) In all groups, constancy of sodium excretion rates during the three control periods was required. The latter was defined as a change in absolute Na excretion ($U_{Na}V$) of no more than 20% in either direction between the first and third control period.

Statistical analyses were performed in the following manner. When experimental results were compared with control data obtained in individual rats such that the same kidney served as its own control, mean values for the respective sets of clearance periods were compared using a paired *t* test. When comparison was made between the two kidneys of the same animal, significance also was determined using a paired *t* test. When the experimental results between groups were compared, an unpaired *t* test was employed.

Results

Figure 1 depicts the results of two representative experiments in stage I normal hydropenic rats. In one of these, a natriuretic fraction was infused into the left renal artery; in the other, a nonnatriuretic factor was infused. In both experiments, values for FE_{Na}, $U_{Na}V$ and GFR were obtained for the infused and contralateral kidneys. The uremic fraction produced a significant increase in both FE_{Na} and $U_{Na}V$ in the infused kidney, and values for both returned towards control levels in the follow-up periods. GFR remained stable throughout. The contralateral kidney was unaffected. The nonnatriuretic (normal) fraction produced no changes in FE_{Na}, $U_{Na}V$ or GFR in either kidney.

Uremic fractions. In Table 1, composite data are shown for the six experiments in which natriuretic fractions were infused into the left renal artery of stage I rats. Composite data are also shown in Table 1 for the six experiments in which the same fractions were infused into the renal arteries of six stage III rats [blood urea nitrogen (BUN) concentration, 64.5 ±

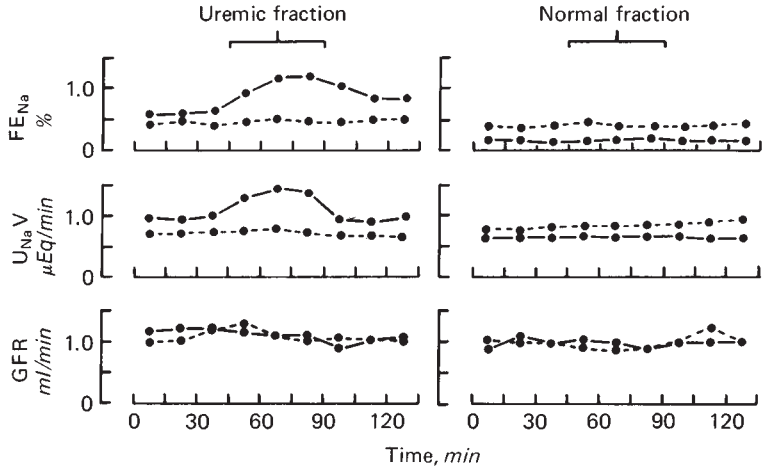

Fig. 1. *Effects of uremic vs. normal urine fraction on FE_{Na}, $U_{Na}V$ and GFR, in two typical experiments.* The *solid line* indicates values for the left (infused) kidney. The *dotted line* indicates values for the right (noninfused kidney). The experimental period was preceded and followed by control periods during which 0.9% NaCl containing 4 mEq/liter of KCl was infused. Infusion rate was 20 μl/min throughout.

4.2 mg/100 ml]. Finally, data are included in Table 1 for the experiments in which three of these uremic fractions were infused into the renal artery of the remnant kidneys of stage II rats.

Normal (stage I) rats. In the stage I rats, the values for both $\Delta U_{Na}V$ and ΔFE_{Na} were statistically significant in the infused organs, (+ 0.87 μEq/min and + 0.60%, respectively). GFR did not change. No changes in values for any of the parameters were observed in the contralateral noninfused kidneys. No significant changes were observed in blood pressure, hematocrit, C_{PAH}, plasma sodium or potassium concentrations or potassium excretion.

Uremic (stage III) rats. In the stage III rats, the values for $\Delta U_{Na}V$ (+ 1.90μEq/min) and ΔFE_{Na} (+ 1.85%) were not only significant in relation to the control values in the same animals, but they were significantly greater than the comparable increments in sodium excretion observed in the stage I rats receiving the same fractions. The stage III animals also failed to show any significant changes in blood pressure, hematocrit, C_{PAH}, plasma Na and K concentrations, or potassium excretion.

In both the normal stage I rats and the uremic stage III rats, the natriuresis induced by the fractions was apparent during the first 15-min experimental

Table 1. Fractions from uremic patients infused (a) into the left renal artery of stage I (normal) rats, (b) into the solitary renal artery of stage III (uremic) rats with a single remnant kidney, and (c) into the remnant kidney of stage II rats (intact kidney plus remnant kidney): effects on glomerular filtration rate and sodium excretion

		(a) Stage I rats (left kidney)			(b) Stage III rats (remnant kidney)			(c) Stage II rats (remnant kidney)		
Fraction		C_{In} *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %	C_{In} *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %	C_{In} *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %
CO	Control	1.37	0.49	0.23	0.68	0.53	0.57	0.65	0.51	0.51
	Δ	+0.02	+0.57	+0.23	−0.13	+1.26	+1.84	−0.01	+0.13	+0.34
AP	Control	1.42	1.38	0.66	0.35	0.50	1.08	—	—	—
	Δ	−0.06	+1.13	+0.52	+0.05	+0.60	+0.94	—	—	—
JO	Control	0.97	0.63	0.43	0.51	0.91	1.36	—	—	—
	Δ	−0.15	+0.70	+0.68	+0.16	+3.10	+2.72	—	—	—
PO	Control	1.30	1.06	0.60	0.69	1.90	1.80	0.31	0.25	0.91
	Δ	−0.18	+0.69	+0.53	+0.05	+1.41	+1.23	−0.02	+0.26	+0.61
LU	Control	1.41	0.47	0.24	0.75	3.07	2.92	—	—	—
	Δ	−0.32	+0.94	+0.64	+0.01	+3.42	+2.48	—	—	—
DI	Control	1.65	0.37	0.10	0.44	0.38	0.91	0.65	0.38	0.41
	Δ	−0.62	+1.22	+1.0	+0.02	+1.61	+1.90	+0.11	+0.27	+0.43
Mean control ± SEM		1.35±0.09	0.73±0.16	0.38±0.09	0.57±0.06	1.21±0.43	1.44±0.34	0.53±0.11	0.38±0.08	0.61±0.15
Mean Δ ± SEM		−0.18±0.12	0.87±0.11	+0.60±0.10	+0.04±0.04	+1.90±0.45	1.85±0.28	+0.03±0.04	+0.22±0.05	0.46±0.08
P exp. vs. control		NS	<0.001	<0.0025	NS	<0.01	<0.005	NS	<0.025	<0.025
P stage I vs. stage II				<0.05						<0.0005

period. The values for $\Delta U_{Na}V$ and ΔFE_{Na} (comparing the results of the first experimental period with the mean of the three control periods) were statistically significant in both groups of animals. Moreover, the magnitude of the natriuresis during the first experimental period was significantly greater in the stage III rats ($\Delta U_{Na}V$, 1.41 μEq/min; FE_{Na}, 1.34%) than in the stage I rats ($\Delta U_{Na}V$, 0.62 μEq/min; ΔFE_{Na}, 0.46%) ($P < 0.025$).

Nonuremic (stage II) rats with one normal and one remnant kidney. For the three stage II rats in which the natriuretic fractions were infused into the renal artery of the remnant kidney (Table 1), the BUN averaged 37.0 ± 4.2 mg/100 ml. The mean control value for GFR in the remnant kidneys of the stage II rats (0.53 ± 0.11 ml/min) was closely comparable to the control GFR for the remnant kidney of the stage III rats receiving the same fractions (0.6 ± 0.08 ml/min). Values for both $U_{Na}V$ and FE_{Na} increased in the remnant kidneys, but the increments, though statistically significant, were small in magnitude ($\Delta U_{Na}V$ = +0.22 μEq/min, and ΔFE_{Na} = +0.46%). No changes occurred in sodium excretion in the contralateral, noninfused kidneys. In comparing the response of the stage II remnant kidneys with the stage III remnant kidneys, in which the same natriuretic fractions were employed, the increments in both $U_{Na}V$ and FE_{Na} were significantly greater in the latter group ($P < 0.001$ and $P < 0.005$, respectively). Moreover, the values for both $\Delta U_{Na}V$ and ΔFE_{Na} in the remnant kidneys of the stage II rats were not significantly different from those observed in the stage I rats receiving the identical natriuretic fractions.

In order to compare further remnant and normal kidneys in identical (nonuremic) environments and at comparable baseline fractional sodium excretion rates, five uremic fractions were administered by i.v. infusion into salt-loaded stage II rats. The results of this maneuver are shown in Table 2. Control values for GFR and $U_{Na}V$ were greater in the intact than the remnant kidney ($P < 0.001$ and $P < 0.025$, respectively); however, FE_{Na} was not significantly different between the two kidneys. Infusion of the fractions caused a small decrease in GFR in the intact kidney and led to a significant increase in $U_{Na}V$ and FE_{Na} in both kidneys. $\Delta U_{Na}V$ was significantly greater for the intact than the remnant kidney, whereas ΔFE_{Na} was not significantly different between the two.

Rats with left renal denervation. The results of the infusion of the natriuretic fractions into the renal arteries of denevervated kidneys of seven stage I rats are shown in Table 3. In the noninfused normal contralateral kidneys (not shown in the table), the control value for GFR was 1.12 ± 0.04 ml/min; for $U_{Na}V$, 0.52 ± 0.20 μEq/min; and for FE_{Na}, 0.39 ± 0.5%. Control values for $U_{Na}V$ and FE_{Na} were significantly greater in the denervated than in the contralateral nondenervated kidney. A substantial increase was produced by the natriuretic fractions in both $U_{Na}V$ (1.62 μEq/min; $P < 0.0025$) and FE_{Na} (1.14%; $P < 0.005$), representing increments of 42% and 45%, respectively. There were no significant differences in GFR between the left and right kidneys either during the control or the experimental clearance period. No changes in $U_{Na}V$ or FE_{Na} occurred in the noninfused normal contralateral kidneys. A comparison of the effects of four fractions (CO, JO, LU, DI) infused into normal kidneys (Table 1) and

Table 2. Fractions from uremic patients administered intravenously to stage II rats with one intact and one remnant kidney: Effects on glomerular filtration rate and sodium excretion

Fraction		Intact kidney			Remnant kidney		
		GFR *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %	GFR *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %
RO	Control	1.89	2.98	1.16	0.43	1.76	3.12
	Δ	−0.42	+1.79	+1.34	−0.09	+1.16	+3.65
PO	Control	2.18	1.72	0.71	1.03	1.79	1.36
	Δ	−0.07	+2.86	+0.98	−0.09	+0.97	+1.13
GU	Control	1.81	2.58	1.17	0.65	0.91	1.16
	Δ	−0.07	+3.71	+1.75	−0.03	+1.11	+1.48
CO	Control	1.99	3.87	1.25	0.65	1.00	1.25
	Δ	−0.22	+2.48	+1.13	−0.02	+0.50	+0.77
HO	Control	1.95	3.44	1.50	0.33	1.16	2.52
	Δ	−0.07	+2.82	+1.07	−0.01	+0.25	+0.74
Mean Control ± SEM		1.96 ± 0.06	2.92 ± 0.37	1.16 ± 0.13	0.62 ± 0.12	1.32 ± 0.19	1.88 ± 0.40
Mean Δ ± SEM		−0.17 ± 0.06	+2.73 ± 0.31	+1.25 ± 0.14	−0.05 ± 0.02	+0.80 ± 0.18	1.55 ± 0.54
P exp. vs. control		<0.05	<0.0005	<0.0005	NS	<0.01	<0.025
P intact vs. remnant		NS					
			<0.0005				
				NS			

Table 3. Fractions from uremic subjects infused into (a) the left renal artery of rats with left renal denervation and (b) the solitary renal artery of stage III rats with remnant kidneys: Effects on glumerular filtration rate and sodium excretion

Fraction		(a) Denervated kidneys			(b) Remnant kidneys		
		GFR *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %	GFR *ml/min*	$U_{Na}V$ *μEq/min*	FE_{Na} %
CO	Control	1.08	2.48	1.64	0.68	0.53	0.57
	Δ	+0.08	+2.55	+1.39	−0.13	+1.26	+1.84
JO	Control	1.23	8.12	5.52	0.51	0.91	1.36
	Δ	−0.09	+0.97	+1.16	+0.16	+3.10	+2.72
LU	Control	1.14	3.54	2.21	0.75	3.07	2.92
	Δ	+0.13	+1.73	+0.92	+0.01	+3.42	+2.48
DI	Control	0.96	3.72	2.75	0.44	0.38	0.91
	Δ	−0.01	+2.53	+2.02	+0.02	+1.61	+1.90
TB	Control	1.08	2.07	1.20	0.35	0.93	1.81
	Δ	−0.01	+0.75	+0.64	−0.01	+1.25	+2.37
AL	Control	1.33	3.13	1.68	0.64	0.75	0.80
	Δ	+0.01	+1.01	+0.76	+0.05	+1.00	+1.01
SM	Control	0.98	3.69	2.59	0.53	0.80	1.08
	Δ	+0.06	+1.82	+1.06	−0.02	+1.30	+1.86
Mean control ± SEM		1.11 ± 0.05	3.82 ± 0.75	2.51 ± 0.54	0.56 ± 0.05	1.10 ± 0.40	1.35 ± 0.30
Mean Δ ± SEM		+0.02 ± 0.03	+1.62 ± 0.28	+1.14 ± 0.18	+0.01 ± 0.03	+1.85 ± 0.37	+2.03 ± 0.21
P exp. vs. control		NS	<0.0025	<0.0005	NS	<0.0025	<0.0005
P Δdenerv. vs. Δremnant		NS					
			NS				
				<0.005			

denervated kidneys (Table 3) reveals a significantly greater increase in both $U_{Na}V$ ($P < 0.025$) and FE_{Na} ($P < 0.025$) in the denervated organs.

Table 3 also compares the effects of the same seven uremic fractions infused into stage III (remnant) kidneys with those observed in the denervated kidneys. The mean control GFR was significantly lower in the stage III kidneys ($P < 0.0005$); control values for both $U_{Na}V$ and FE_{Na} were also significantly lower in the remnant kidneys than in the denervated kidneys ($P < 0.005$ and $P < 0.05$, respectively). Despite these differences $U_{Na}V$ increased in the remnant kidneys by 1.85 μEq/min, an increment which was not different from that observed in denervated kidneys with approximately four times the number of nephrons; FE_{Na} increased by 2.03% which was significantly greater than that observed in the denervated kidneys ($P < 0.005$). Expressed as percentage over control values, $U_{Na}V$ increased by 168% and FE_{Na} by 150% in remnant kidneys. Both these increments are significantly greater than the percentage increments of 42 and 45% observed in the denervated kidneys ($P < 0.0025$).

Nonnatriuretic Fractions. The results of the studies using nonnatriuretic fractions from normal subjects are shown in Table 4. In none of the three experimental groups (stage I rats, stage I rats with left renal denervation and stage III rats) was there a significant change in $U_{Na}V$, FE_{Na} or GFR. The lack of any significant effects applied both to intragroup comparisons (infused vs. contralateral noninfused kidneys) in the stage I and stage I denervated groups, and to intergroup comparisons. No significant changes were observed in any of the groups in blood pressure, hematocrit, C_{PAH}, plasma Na or K concentrations and potassium excretion.

Discussion

The evidence that the natriuretic factor employed in the present studies is linked to the patterns of sodium excretion stems from several experimental observations. *1*) The factor is detectable in the serum and urine of uremic patients with high rates of fractional sodium excretion, whereas it is not detectable in comparable fractions obtained from normal subjects with low values for FE_{Na} [5, 6]. *2*) In dogs rendered uremic by sequential reduction of renal mass and fed a constant sodium intake, FE_{Na} rose as GFR fell, and at high values for FE_{Na}, natriuretic factor was detectable. In a second group of dogs subjected to identical reduction of renal mass, sodium intake was decreased in exact proportion to the fall in GFR. In these animals, FE_{Na} remained at control levels (despite the presence of uremia), and natriuretic activity was not detectable [7]. *3*) In patients with advanced uremia who have coexisting nephrotic syndrome and are nonnatriuretic edema-formers with *low* values for FE_{Na}, natriuretic activity in serum and/or urine fractions was not detectable [6]. *4*) The factor recently has been demonstrated in urine of normal dogs on a high salt intake and a mineralocorticoid undergoing "escape" [8]. *5*) Finally, the factor recently has been demonstrated in

Table 4. Fractions from normal subjects infused into the left renal artery of (a) normal (stage I) rats, (b) normal rats with left renal denervation and a contralateral intact kidney and (c) uremic rats with a solitary remnant kidney: Effects on glomerular filtration rate and sodium excretion

Fraction		C_{In} ml/min		$U_{Na}V$ μEq/min		FE_{Na} %	
		Right	Left	Right	Left	Right	Left
				a. Normal rats			
GR	Control	1.13	1.38	0.09	0.12	0.06	0.07
	Δ	+0.31	−0.39	+0.05	−0.01	+0.01	+0.02
DR	Control	1.60	1.60	2.51	2.80	1.10	1.23
	Δ	+0.23	−0.03	+0.11	+0.14	−0.10	+0.09
KA	Control	1.00	0.96	0.60	0.16	0.43	0.12
	Δ	−0.10	+0.05	+0.04	0	+0.09	+0.01
FO	Control	1.28	1.35	0.38	0.44	0.35	0.24
	Δ	−0.45	+0.23	+0.04	+0.07	−0.12	+0.18
SK	Control	1.53	1.61	1.09	0.95	0.49	0.40
	Δ	+0.17	+0.11	+0.69	+0.75	+0.23	+0.23
Mean control ± SEM		1.31 ± 0.09	1.39 ± 0.12	0.93 ± 0.43	0.90 ± 0.50	0.48 ± 0.17	0.41 ± 0.21
Mean Δ ± SEM		+0.03 ± 0.14	−0.01 ± 0.10	+0.19 ± 0.14	+0.19 ± 0.14	+0.10 ± 0.04	+0.11 ± 0.15
P exp. vs. control		NS	NS	NS	NS	NS	NS
				b. Left renal denervation			
GR	Control	0.89	1.02	0.97	7.31	0.81	5.23
	Δ	−0.04	−0.09	+0.38	−0.25	+0.34	+0.23
DR	Control	1.35	1.51	0.46	3.38	0.24	1.59
	Δ	+0.17	−0.12	0.22	+1.21	+0.07	+0.67
KA	Control	1.44	0.97	1.03	4.05	0.53	3.02
	Δ	+0.23	−0.15	0.36	−0.94	+0.07	−0.37
FO	Control	1.12	1.05	0.86	1.53	0.54	1.04
	Δ	1.12	1.13	−0.10	−0.13	−0.05	−0.15
Mean control ± SEM		1.20 ± 0.12	1.14 ± 0.13	0.83 ± 0.13	4.07 ± 1.20	0.53 ± 0.12	2.72 ± 0.93
Mean Δ ± SEM		+0.09 ± 0.06	−0.07 ± 0.05	+0.22 ± 0.11	−0.03 ± 0.45	+0.11 ± 0.08	+0.09 ± 0.23
P exp. vs. control		NS	NS	NS	NS	NS	NS
				c. Uremic (stage III) rats			
LU	Control	0.76		2.73		2.29	
	Exp. Δ	+0.15		−0.78		−0.58	
MB	Control	0.96		6.65		4.68	
	Exp. Δ	−0.01		+0.57		+0.54	
ZA	Control	0.86		1.04		0.82	
	Exp. Δ	−0.07		+0.1		+0.20	
FL	Control	0.52		0.74		1.04	
	Exp. Δ	+0.10		+0.38		+0.26	
JR	Control	0.81		0.50		4.60	
	Exp. Δ	−0.08		−0.67		−0.23	
Mean Control ± SEM		0.78 ± 0.07		3.23 ± 1.14		2.68 ± 0.84	
Mean Δ ± SEM		0.02 ± 0.05		−0.08 ± 0.27		+0.04 ± 0.20	
P exp. vs. control		NS		NS		NS	

normal young men undergoing "water immersion" natriuresis [9].

In all of the foregoing states, the evidence suggests that natriuretic factor appears to be produced in increased amounts and/or that its biologic half-life is prolonged. This evidence consists of the finding of a greater activity of the natriuretic factor in the serum and the simultaneous excretion of a greater quantity of natriuretic factor in the urine than is found in nonnatriuretic animals and man. A number of other investigators have demonstrated the presence of natriuretic factor(s) or inhibitors of Na transport in the blood or urine of normal animals and man [11–17].

The present studies were designed to examine the possibility that the sensitivity of the nephron to the biologic effects of the natriuretic factor increases as the total nephron population and GFR diminish.

In normal hydropenic rats with a low baseline level of FE_{Na}, infusion of the natriuretic factor into one renal artery produced a small but statistically significant increase in both $U_{Na}V$ and FE_{Na} in the infused kidney only, with no changes occurring in the contralateral organ. When the same quantity of the same natriuretic fractions was infused into the renal artery of the remnant kidney of stage III uremic rats, the increase in both $U_{Na}V$ and FE_{Na} was significantly greater than that observed in the stage I animals. The fact that values for $\Delta U_{Na}V$ were greater in stage III than stage I rats is especially significant in view of the fact that the infused kidneys in the latter group had

approximately four to five times as many nephrons as in the former group.

The possibility was considered that this augmented natriuresis could be due to the administration of the same amount of the fraction to a smaller mass of renal tissue with a diminished total blood flow leading to the delivery of higher concentrations to the remaining nephrons. When, however, the same volumes of the fractions were infused into the remnant kidneys of stage II (nonazotemic) animals, the natriuresis that ensued was significantly less than in stage III remnant kidneys with comparable reductions in renal mass and glomerular filtration rates. The possibility that the increased responsivity of the remnant kidney is due to some intrinsic change induced by the reduction in renal mass per se, rather than to an adaptation associated with uremia, is also rendered unlikely by the studies shown in Table 2. When natriuretic factor was infused intravenously into stage II rats, there was no significant difference between the increments in FE_{Na} in the remnant vs. the contralateral normal kidneys. The data thus indicate that the enhanced natriuretic response is associated with some aspect of the uremic state and not to any special characteristics of the remnant kidney or a reduced nephron population per se.

A second possible explanation for the enhanced responsiveness of the uremic rat to the natriuretic factor is that the excretion of the factor is diminished and that the biologic activity persists over a longer period of time. We believe this to be unlikely both because the concentration of the factor in the infusate delivered directly into the renal artery of the remnant kidneys presumably was orders of magnitude greater than the concentration of any of the factor which escaped either binding and/or inactivation or excretion, and which recirculated in the systemic circulation. It was to examine this possibility that comparison was made between the natriuretic response in stage III vs. stage I rats during the first clearance period after initiation of intrarenal infusion of the factor. As indicated previously, the enhanced natriuretic response in the stage III animals was present within the first 15 min of observation.

Recent micropuncture studies [4, 18] have demonstrated a depression of fractional sodium reabsorption in the proximal tubule and an increased delivery of filtrate to more distal nephron sites in uremic animals with remnant kidneys. Since this elevation in delivery could presumably influence the responsiveness to the natriuretic factor, in that the factor has been shown to act on distal nephrons sites [19], an effort also was made to examine, as a separate variable, the influence of baseline levels of FE_{Na} on the natriuretic response using the model of acute renal denervation which has been shown to decrease proximal tubular sodium reabsorption without an effect on GFR [20].

Infusion of the natriuretic factor into denervated kidneys led to a natriuresis which was significantly greater than in the nondenervated normal kidneys of stage I rats, indicating that elevation of baseline sodium excretion enhances the response to the factor. In order to determine whether the augmented response of the remnant kidneys of stage III (uremic) rats could be explained solely by increased control values for sodium excretion, their response to the natriuretic factor was compared with that of the denervated kidneys. Control values for both absolute and fractional sodium excretion were twice as high as in the remnant kidneys yet the absolute increment in sodium excretion was the same in the two groups (despite the fact that the remnant kidneys had one-quarter the number of nephrons). The relative magnitude of the change was, however, far greater in the remnant kidneys (165%) than in the denervated kidneys (42%). The changes in fractional sodium excretion (which relate tubular handling of sodium to the filtered load of sodium) were significantly greater in the remnant kidneys (150% increase) than in the denervated kidneys (45% increase).

It may be argued that changes in FE_{Na} may not be the most pertinent index of responsiveness and that comparisons of absolute increments in sodium excretion are more relevant. On the other hand, if control values for FE_{Na} in denervated and remnant kidneys are comparable, it is implicit that the ratio between tubular rejection of sodium and filtered sodium must be comparable. Since changes in the filtered load did not occur in any of the present experiments, an increment in FE_{Na} can only be due to an alteration in tubular reabsorption. The greater increment, seen in the uremic remnant kidneys as compared with the denervated kidneys, can only be interpreted as indicating an enhanced tubular responsiveness to the natriuretic factor which is not specifically dependent on the baseline conditions. This conclusion is further supported by the finding that absolute sodium excretion underwent a percentage increase over control which was three times greater than in the denervated kidneys.

These data indicate that the baseline rate of FE_{Na}, and presumably distal sodium delivery, condition the natriuretic response to the natriuretic factor. However, the enhanced responsivity of the residual nephrons in the stage III rats seems also to reflect a true adaptive increase in end-organ sensitivity to the factor.

Acknowledgements

This study was supported by Public Health Service program project grant PO 1 Am16281 and training grant 5T01 HL05928 from the National Institutes of Health, and by training grant C-72074 from the New York State Kidney Disease Institute. Dr. Bourgoignie was recipient of Public Health Service Research Career Development Award 7 KO 4 HL40977. Dr. K. Hwang and Tom Dreager assisted in the laboratory, and Pat Kanakos and Ruth Glick provided secretarial assistance.

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